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Osilodrostat (LCI699), a potent 11β-hydroxylase inhibitor, administered in combination with the multireceptor-targeted somatostatin analog pasireotide: A 13-week study in rats

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ABSTRACT

The somatostatin analog pasireotide and the 11β-hydroxylase inhibitor osilodrostat (LCI699) reduce cortisol levels by distinct mechanisms of action. There exists a scientific rationale to investigate the clinical efficacy of these two agents in combination. This manuscript reports the results of a toxicology study in rats, evaluating different doses of osilodrostat and pasireotide alone and in combination. Sixty male and 60 female rats were randomized into single-sex groups to receive daily doses of pasireotide (0.3 mg/kg/day, subcutaneously), osilodrostat (20 mg/kg/day, orally), osilodrostat/pasireotide in combination (low dose, 1.5/0.03 mg/kg/day; mid-dose, 5/0.1 mg/kg/day; or high dose, 20/0.3 mg/kg/day), or vehicle for 13 weeks. Mean body-weight gains from baseline to Week 13 were significantly lower in the pasireotide-alone and combined-treatment groups compared to controls, and were significantly higher in female rats receiving osilodrostat monotherapy. Osilodrostat and pasireotide monotherapies were associated with significant changes in the histology and mean weights of the pituitary and adrenal glands, liver, and ovary/oviduct. Osilodrostat alone was associated with adrenocortical hypertrophy and hepatocellular hypertrophy. In combination, osilodrostat/pasireotide did not exacerbate any target organ changes and ameliorated the liver and adrenal gland changes observed with monotherapy. C_{max} and AUC_{0-24h} of osilodrostat and pasireotide increased in an approximately dose-proportional manner.

In conclusion, the pasireotide and osilodrostat combination did not exacerbate changes in target organ weight or toxicity compared with either monotherapy, and had an acceptable safety profile; addition of pasireotide to the osilodrostat regimen may attenuate potential adrenal gland hyperactivation and hepatocellular hypertrophy, which are potential side effects of osilodrostat monotherapy.

Introduction

The investigational oral agent osilodrostat (LCI699) is a potent inhibitor of human 11β-hydroxylase (CYP11B1; IC_{50} = 2.5 nM) and aldosterone synthase (CYP11B2; IC_{50} = 0.7 nM), which are responsible for catalyzing the final steps of cortisol and aldosterone biosynthesis, respectively, in the adrenal cortex (Bassett et al., 2004; Menard et al., 2010; Calhoun et al., 2011). Given its inhibition of the cortisol biosynthesis pathway, osilodrostat is currently under investigation as a potential new treatment option for Cushing's syndrome, a rare disorder of chronic hypercortisolism, and has shown encouraging efficacy and safety in a proof-of-concept study of 12 patients with Cushing's disease (Bertagna et al., 2014).

Pasireotide is a multireceptor-targeted somatostatin analog with high binding affinity to four of the five somatostatin receptor subtypes ($sst_{1,2,3}$ and sst_5), with an IC_{50} of 9.3, 1.0, 1.5, and 0.16 nM for human sst_1, sst_2, sst_3, and sst_5, respectively (Bruns et al., 2002). Activation of these receptor subtypes, especially sst_5, by pasireotide at the pituitary gland potently suppresses adrenocorticotropic hormone (ACTH) and growth hormone (GH) secretion (Colao et al., 2012, 2014). ACTH is an important component of the hypothalamic–pituitary–adrenal (HPA) axis, which stimulates corticosteroid production by the adrenal glands. In a large phase III trial, subcutaneous (sc) administration of pasireotide

Abbreviations: ACTH, adrenocorticotropic hormone; GH, growth hormone; HPA, hypothalamic–pituitary–adrenal axis; IGF-1, insulin-like growth factor 1; sc, subcutaneous; sst, somatostatin receptor subtypes.

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Table 1
Drug doses applied by randomized group.

Group	Osilodrostat/pasireotide dose level (mg/kg/day)	Males (*n*)	Females (*n*)
Vehicle control	0/0	10	10
Low-dose Osilodrostat/pasireotide	1.5/0.03	10	10
Mid-dose Osilodrostat/pasireotide	5/0.1	10	10
High-dose Osilodrostat/pasireotide	20/0.3	10	10
High-dose osilodrostat	20/–	10	10
High-dose pasireotide	–/0.3	10	10

led to a rapid and robust reduction in mean urinary free cortisol levels that was maintained over time (Colao et al., 2012). Based on the results of this study, a twice-daily sc formulation of pasireotide (Signifor®) has been approved for the treatment of patients with Cushing's disease in the United States, Europe, and several other countries (PI, 2012; SPC, 2013).

Pasireotide and osilodrostat have been shown to reduce cortisol levels through distinct mechanisms of action at different levels of the HPA axis. Pasireotide inhibits ACTH secretion from the pituitary gland, while osilodrostat inhibits the biosynthesis of cortisol in the adrenal cortex (Bertagna et al., 2014). Thus, there is a scientific rationale for investigating the efficacy of these 2 agents in combination. As the safety profile of this drug combination has not been established, it is initially necessary to examine the toxicity profile and potential drug–drug interactions associated with the coadministration of osilodrostat and pasireotide. This paper reports the results of a 13-week toxicology study with different doses of osilodrostat and pasireotide, alone and in combination, in Wistar rats.

Methods

The experiments described in this paper were performed in accordance with the National Animal Welfare guidelines of the US National Research Council and the Canadian Council on Animal Care. Good Laboratory Practice regulations were observed.

Study design. Sixty male and 60 female rats were randomized into single-sex groups to receive daily doses of vehicle, low-dose osilodrostat/pasireotide, mid-dose osilodrostat/pasireotide, high-dose osilodrostat/pasireotide, high-dose osilodrostat monotherapy, or high-dose pasireotide monotherapy (Table 1). Prior to initiation of dosing, all animals were weighed, and randomization was stratified by body weight. Rats received their specified treatment regimen daily for 13 weeks; the 13-week treatment period was chosen according to current European Medicines Agency recommendations (ICH M3 [R2]). Three female sentinel rats were used for health screening procedures.

Osilodrostat was administered orally using a plastic gavage tube, followed by, where applicable, sc injection of pasireotide into the interscapular area within 5 min of osilodrostat administration. Animals were dosed at approximately the same time each day, except during designated procedures. Oral and sc routes of administration were selected for osilodrostat and pasireotide, respectively, as they represent the intended routes of administration in humans.

The low, mid, and high doses of osilodrostat (1.5, 5, and 20 mg/kg/day) and pasireotide (0.03, 0.1, and 0.3 mg/kg/day) were considered appropriate based on the results of previous monotherapy studies in rats (osilodrostat dose range: 0.2–50 mg/kg/day, orally; pasireotide dose range: 0.08–0.24 mg/kg/day, sc; Novartis Pharma AG, unpublished data); these doses were expected to provide sufficient exposure multiples against human systemic exposure at therapeutic doses. Osilodrostat and pasireotide doses of up to 20 and 0.24 mg/kg/day, respectively, were tolerated during two 6-month monotherapy studies; the no-observed-adverse-effect levels (NOAELs) for osilodrostat and pasireotide were 2 and 0.024 mg/kg/day, respectively (Novartis Pharma AG, unpublished data).

Compounds and formulation. Osilodrostat was formulated in ultrapure water for administration by oral gavage. Pasireotide was formulated with acetate-buffered solution (pH 4.5), acetic acid, and D-mannitol in sterile water for sc injection. Vehicle control consisted of ultrapure water for oral gavage and acetate-buffered solution (pH 4.5), acetic acid, and D-mannitol in sterile water for sc injection. Dosing volumes were 5 mL/kg for oral gavage (osilodrostat and vehicle) and 1 mL/kg for sc injection (pasireotide and vehicle). Agents were stored at 4 °C and protected from light. For administration of osilodrostat, dosing solutions were removed from the refrigerator and stirred for at least 10 min at room temperature prior to initiation of dosing. Dosing solutions of pasireotide were removed from the refrigerator and allowed to warm to room temperature for at least 30 min prior to initiation of dosing.

Animals and housing. Rattus norvegicus, Wistar Hannover Crl:WI (Han), were 7–8 weeks of age and had body weights of 176–216 g for males and 123–165 g for females at the start of dosing. Rats were housed with up to 2 other animals of the same sex and dosing group in polycarbonate bins containing appropriate bedding and equipped with an automatic watering valve. A 12-hour light/dark cycle was in effect, and rats were housed at a room temperature of 19–25 °C with relative humidity of 30–70%. Food (PMI Nutrition International Certified Rodent Chow No. 5CR4 [14% protein]) and municipal tap water that had been softened, purified by reverse osmosis, and exposed to ultraviolet light were provided *ad libitum*. There were no known contaminants in the food or water that interfered with the conduct of the study.

Endpoints and assessments. This study sought to determine the effects of daily osilodrostat and pasireotide, alone and in combination, on the pituitary–adrenal axis and to report any other treatment-related toxicities. The toxicokinetic characteristics of osilodrostat and pasireotide were also determined.

The schedule of study assessments is summarized in Table 2. Cage-side observations were performed daily pre-dose and 1 h post-dose. Evaluation of body weight and food consumption was performed at baseline and weekly thereafter. Ophthalmologic evaluations were

Table 2
Schedule of endpoint collections and study timeline.

Week	−1	1	2	3	4	5	6	7	8	9	10	11	12	13	Day 92
Cage-side observations	Daily (pre-dose and 1 h post-dose)														
Body weight[a]	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Food consumption	X	x	x	x	x	x	x	x	x	x	x	x	x	x	
Ophthalmology	X													x	
Clinical pathology (hematology, serum chemistry, urinalysis)					x										x
IGF-1															x
Toxicokinetic evaluations		x										x			
Euthanasia[b]															x

[a] Body weight was measured prior to randomization, on Day −1, and weekly thereafter.
[b] Necropsy, tissue collection, and organ weight measurements were performed on Day 92.